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SYNOPTIC NOTE

For the degree of

Doctor of Philosophy

In

"Evaluation of cardioprotective action of cucurbitacin conjugated magnesium nanoparticles through transcriptomics approach"

By

Ms. Vidyashree Suryavanshi

Under the Guidance of

Dr. Joy Hoskeri

Assistant Professor

Department of Bioinformatics

Karnataka State Akkamahadevi Women University,

Vijayapura.

Abstract

Sinoatrial node and atrioventricular node are the special muscle tissues that also act as neurons. Sinoatrial node is the node where impulses are received from peripheral nervous system. Later the impulses are sent to atrioventricular node, then to all the purkinje's fibrils. Many agaents such as amitriptyline or isoproterenol are proven as cardiotoxic to humans. It directly or indirectly enters into human body and causes severe damage to all the organs of the body including heart. Hence, in this work, we shall investigate the cucurbitacin conjugated magnesium nanoparticles protectivity against cardiotoxicity. We shall use H9C2 cell lines and rat models for the testing of protective effects of the cucurbitacin conjugated magnesium nanoparticles. In investigation will cover synthesis of magnesium oxide nanoparticles and conjugation of MgO with cucurbitacin. Further cardioprotective mechanism of cucurbitacin conjugated magnesium nanoparticles will be unmasked by Biochemical, histopathological, cardiophysiological studies, transcriptomics analysis of heart tissue and bioinformatics studies focusing on network pharmacology.

Key words: Cardiovascular disease; Magnesium nanoparticles; Transcriptomics; Cardiotoxicity; Amitriptyline; Sinoatrial node; Atrioventricular node; Bioinformatics.

Introduction

Cardiovascular diseases

Cardiovascular disease (CVD) is a general term that depicts a sickness of the heart or veins. It's generally associated with a development of greasy stores inside the conduits (atherosclerosis) and an expanded gamble of blood clusters.

It can likewise be related with harm to supply routes in organs like the cerebrum, heart, kidneys and eyes. CVD is one of the primary drivers of death and handicap in the UK, however it can frequently to a great extent be forestalled by driving a sound way of life.

There are various kinds of CVD, among them the primary kinds are,

1) Coronary diseases - Coronary illness happens when the progression of oxygen-rich blood to the heart muscle is impeded or diminished. 2) Strokes and TIAS - A stroke is where the blood supply to some portion of the mind is cut off, which can cause cerebrum harm and conceivably passing. 3) Fringe blood vessel diseases - Fringe blood vessel illness happens when there's a blockage in the veins to the appendages, generally the legs. 4) Aortic diseases - Aortic illnesses are a gathering of conditions influencing the aorta. This is the biggest vein in the body, which conveys blood from the heart to the remainder of the body.

Cardiovascular diseases (CVDs) are the significant reason for death worldwide. An expected 17.9 million individuals kicked the bucket from CVDs in 2019, introducing 32% of every single worldwide passing. Of these passings, 85% were because of respiratory failure and stroke. North of 3/4 of CVD passings happen in low-and center pay countries. Out of the 17 million early passings (younger than 70) because of non-transmittable sicknesses in 2019, 38% were brought about by CVDs. Most cardiovascular illnesses can be forestalled by tending to conduct risk factors, for example, tobacco use, undesirable eating regimen and corpulence, actual latency and unsafe utilization of liquor. It is critical to distinguish cardiovascular infection as soon as conceivable so the board with guiding and medications can start (World health Organisation).

The sinoatrial node is an oval shaped locale of extraordinary heart muscle in the upper back mass of the right chamber comprised of cells known as pacemaker cells. These cells can create an electrical motivation known as a cardiovascular activity expected that movements through the

electrical conduction arrangement of the heart, making it contract. In a solid heart, the SA hub persistently delivers activity possibilities, setting the cadence of the heart (sinus beat), as is known as the heart's normal pacemaker. Sinus node dysfunction is frequently brought about by abnormal pacemaker cell impulse production or inappropriate conduction across the perinodal cells. The acquired form is more common; it can also be inherited. Patients could or might not have symptoms. (Kashou et al., 2017). A series of irregular heartbeat diseases called sinus node dysfunction, commonly referred to as sick sinus syndrome, are brought on by incorrect electrical signals in the heart. The heart's rhythms become aberrant when the sinoatrial node is damaged; they are often too slow, exhibit pauses in function, or a mix of these, and very rarely are they faster than normal.

Cucurbitacin

The family of plants known as Cucurbitaceae has a variety of different substances known as cucurbitacins. These compounds' medicinal and poisonous qualities have sparked an ongoing interest in them (Kupchan et al., 1978). Tetracyclic triterpenoids that are substantially oxidised are known as cucurbitacins. They are extensively dispersed across the plant world and serve as heterologous chemical pheromones that shield plants from external biological threats. In the 1960s, researchers began to pay attention to the extent of their broad-spectrum pharmacological bioactivities (Chen et al., 2012).

Cucurbitacins have been suggested as the cause of the bitter taste of some plant species, such as cucumber. In 1831, a crystalline form of the first cucurbitacin, known as -elaterin, was discovered. Some plant species, such as *Momordica*, which are abundant in cucurbitacins, are highly prized in various systems.

Magnesium nanoparticles

Due to their presence as a pollutant in water, air, and food products as byproducts of natural phenomena or due to rising activity in the anthropogenic factors, human exposure to nanoparticles, especially metal-based nanoparticles, grows rapidly. Magnesium nanoparticles are spherical black

high surface area particles typically 20-60 nm in size with specific surface area ranging from 30 to 70 m2/g. These nanoparticles are also available in ultra high purity, high purity, coated and dispersed forms.

Magnesium functions as a cofactor for many enzymes and is crucial for the maintenance of the transmembrane action potential and a high intracellular potassium content. Only 0.3% of the magnesium in the body, which totals roughly 1000 mmol, is found in plasma. Although hypomagnesemia and possible magnesium insufficiency are only seldom accompanied by pertinent clinical symptoms, they are detected in 7 to 11% of hospitalised patients. In cardiology, prolonged diuretic medication and secondary aldosteronism are frequent contributors to hypomagnesemia (Weiss, M. 1995).

Magnesium oxide nanoparticles (Mg nano) are employed as antimicrobial and possible anticancer agents, as well as biocompatible and biodegradable treatments for heartburn, stomach indigestion, and bone regeneration (Dizaj et al., 2014) Despite Mg nano's recent widespread application in the drug delivery sector, nothing is known about NP-mediated toxicity (Khanna et al., 2015).

Amitriptyline

Amitriptyline is FDA endorsed medicine to treat significant burdensome problem (MDD) in adults. the non-FDA-supported signs are uneasiness, post-horrendous pressure problem, sleep deprivation, ongoing agony (diabetic neuropathy, fibromyalgia), crabby gut condition, interstitial cystitis (bladder torment disorder), headache prophylaxis, postherpetic neuralgia, and sialorrhea (Dopheide J A, 2007).

Amitriptyline increments noradrenergic or serotonergic neurotransmission by impeding the norepinephrine or serotonin carrier (net or sert) at presynaptic terminals. Persistent treatment with amitriptyline desensitizes presynaptic autoreceptors and heteroreceptors, delivering dependable changes in monoaminergic neurotransmission. It is really calming and has expanded anticholinergic properties contrasted with other TCAS. Like different antidepressants, the beginning of restorative activity normally starts at roughly 2 to about a month (Bowden et al.,

1985). Measurement definitions: amitriptyline dose details come in oral tablets of 10 mg, 25 mg, 50 mg, 75 mg, 100 mg, and 150 mg (https://www.medicalnewstoday.com/articles/amitriptyline-oral-tablet).

Tricyclic antidepressants (TCAs) are commonly used for many different psychiatric and nonpsychiatric disorders. Intentional overdose for suicide with these agents is common, resulting in substantial morbidity and mortality owing to ventricular tachyarrhythmias (Biggs et al., 1977; Giardina et al., 1979; Marshall & Forker, 1982) Amitriptyline, a TCA that is commonly used to treat manic depression, has been demonstrated to lengthen the QT interval and to cause torsades de pointes in human hearts (Nishimoto et al., 1994; Alderton, 1995). The electrophysiological foundation for the drug's arrhythmogenic actions hasn't been identified, though.

Meta-analysis

Glass first defined meta-analysis in the social science literature as "The statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings" (Glass GV, 1976).

A quantitative, formal, epidemiological study method known as a "meta-analysis" is used to carefully evaluate the findings of earlier research in order to draw conclusions about that body of work. The study is typically—but not always—based on randomised, controlled clinical trials. Results from a meta-analysis may be more accurate estimates of the impact of a treatment, a disease risk factor, or other outcomes than those from any one study that contributed to the pooled analysis. Examining the heterogeneity of a collection of studies and the generalizability of responses can help identify sources of variation in responses, which can help develop more effective therapies or management changes (Haidich, 2010).

Transcriptomics

Transcriptomics is the investigation of the 'transcriptome,' a term currently broadly comprehended to mean the total arrangement of all the ribonucleic corrosive (RNA) particles (called records) communicated in some given substance, like a phone, tissue, or living being. Since the late 1990s, when technology began to progress, transcriptomics has become a widely accepted

field of study. The first attempts to examine the entire transcriptome were made in the early 1990s. The area of transcriptomics has been transformed repeatedly by technological advancements. Microarrays, which measure a set of preset sequences, and RNA sequencing (RNA-Seq), which uses high-throughput sequencing to collect all sequences, are two of the most important modern tools in the field (Lowe et al., 2017).

A common genomic method for analysing biological processes is whole-genome transcriptional profiling. Particularly RNA sequencing (RNAseq) has seen a wide range of applications in genetics and many healthcare sectors. Due to declining costs for next-generation sequencing and a lighter computational load for data processing, RNAseq, which requires a relatively straightforward experimental methodology of cDNA library creation and RNA extraction, has emerged as a key technology in contemporary biology. Cell lineage and cell state genetic profiles can now be more precisely characterised because to the recent use of RNAseq methodology in single-cell transcriptional profiling. Analysis of differential gene expression, RNA isoform analysis, haplotype-specific analysis of gene expression (allele-specific expression), and study of expression quantitative trait loci are all made possible by the advent of bioinformatic and statistical methods (Wirka et al., 2018).

OBJECTIVES

- Synthesis and characterization of cucurbitacin conjugated magnesium nanoparticles.
- Evaluation of cardioprotective effects of cucurbitacin conjugated magnesium nanoparticles in *in-vitro* and *in-vivo* system.
- Unmasking the mode of action of cucurbitacin conjugated magnesium nanoparticles through transcriptomics and network analysis.

Review of Literature

Cardiovascular diseases

According to Biswas et al., (2015), the history of sudden death was two times more in obstructive CVD cases as compared to non-obstructive form, specifies the malignant nature of obstructive CVD correlated with poor prognosis. The previous reports justify autosomal dominant mode of inheritance in case of familial CVD, while, the current study states that other modes of inheritance like autosomal recessive may be experienced in a subset of non-obstructive CVD.

We conducted a comprehensive review of studies reporting the association between socioeconomic status (ses) and cvrf, CVD, and CVD-related mortality in India. With the exception of smoking and low fruit and vegetable intake, the studies clearly suggest that cvrf/CVD is more prevalent among high ses groups in india than among the low ses groups. Although CVD-related mortality rates appear to be higher among the lower ses groups, the proportion of deaths from CVD-related causes was found to be greatest among higher ses groups (Subramanian et al., 2013)

Cardiovascular diseases (cvds) have now become the leading cause of mortality in India. A quarter of all mortality is attributable to CVD. Ischemic heart disease and stroke are the predominant causes and are responsible for >80% of CVD deaths. The global burden of disease study estimate of age-standardized CVD death rate of 272 per 100 000 population in India is higher than the global average of 235 per 100 000 population (Prabhakaran et al., 2016).

Chauhan and Aeri (2013) reported that studies show that India has increased acceptance of cardiovascular disease comparing to other developing countries with recent trends that shows prevalence in younger age group. This affect to all the groups of society from young to old and most affluent to least affluent.

Mortality statistics show that CVD remains the most common cause of death in europe, accounting for 45% of all deaths; 49% of deaths among women and 40% among men. More than 4 million people die from CVD across Europe every year, with 1.4 million of these deaths before the age of 75 years. There remains evidence of wide inequalities across Europe in the burden of CVD mortality and the changes in rates of death from these diseases (Townsend et al., 2016).

Yang et al., (2019) have précised the functions and mechanisms of mitochondria derived peptides (mdps) in cardiovascular disease along with their related risk factors, and ended up saying that mdps might be a latest target to treat cardiovascular disease, that will guard myocardial and vascular endothelial cells and maintain cell homeostasis.

New therapeutics and medication targets, for example, serca-2a, aldosterone incorporating chemical, neuroendopeptidases, inhibitors of renin, new targets tweaking capability of adh, at-2r agonists, new targets regulating autonomic sensory system movement will open new windows for the cardiovascular scientists to foster novel more useful medication (Afzal, 2021).

We propose a significant job for type I IFNS in lupus CVD, a speculation that has acquired help in the field of CVD overall. Going ahead, there ought to be proceeded with center around grasping the degree to which explicit restorative regimens — whether currently accessible, similar to antimalarials and MMF, or novel, similar to hostile to IFN — may be especially compelling against this critical reason for dreariness and mortality in patients with sle (Knight and Kaplan, 2013).

The enormous accessibility of GWASS has made a broad development in the disclosure of chance qualities for cardiovascular sickness. Qualities that are recognized by GWASS have affected science and found new pathways or systems, as delineated by the searching for chromosome 9p21/anril, chromosome 1p13/sort1, and — as of late — the chromosome 10q23 lipa locus. In any case, there is an enormous hole remains in view of missing heritability, as extremely less measure of the heritability, for example, creep or hypertension, which is the main cardiovascular sicknesses can be made sense of by the normal hereditary gamble factors recognized by GWASS so far. The field of individual gamble expectation for cardiovascular illness utilizing normal hereditary variables is still in its early stages. Most hereditary gamble factors distinguished so far will show just little chances proportions, delivering the positive prescient upsides of such tests insignificant (Zeller et al., 2012).

According to Poulter, (2003) mediation methodologies that don't focus on those at most elevated cardiovascular gamble are probably going to be less financially savvy. Worldwide gamble assessment, assessing all hazard elements to deliver an evaluation of a singular's outright gamble of chd, is progressively perceived by the board rules as a clinically and practical method for

directing treatment. Notwithstanding, the impediments of utilizing transient outright gamble to decide mediation should be perceived.

Treatment of cardiovascular diseases

Satisfactory nourishment is a component assuming a significant part in the counteraction of civilization related sicknesses. Many mixtures found in food are credited a wide range of wellbeing advancing properties. An illustration of such mixtures might be given via carotenoids. Concentrates on led lately show that they might impact human wellbeing. Research results examined in this paper propose that utilization of items wealthy in carotenoids might add to the restraint of cardiovascular illness improvement. Simultaneously it should be focused on that a few examinations, especially review, don't affirm a worthwhile activity of the examined compounds. It is important to know about the way that a large portion of the mediation concentrates on utilized enhancements of carotenoids instead of their normal sources, which might have impacted. It is important to take note of the way that normal wellsprings of carotenoids likewise contain different parts, for example phytocompounds and nutrients, which might have impacted the examination results (Kulczyński et al., 2017).

Miller (1998) states that A few botanicals, including *Crataegus oxycantha, Terminalia arjuna, Inula racemosa,* and *Astragalus membranaceus*, have been found to have restorative advantage for the treatment of cardiovascular sickness. *Crataegus oxycantha* has been utilized generally as a cardiovascular tonic and current purposes incorporate treatment for angina, hypertension, arrhythmias, and congestive cardiovascular breakdown. In human preliminaries, a mix of *Inula racemosa* and *Commiphora mukul* was demonstrated to be better than dynamite in diminishing the chest agony and dyspnea related with angina. *Astragalus membranaceus,* a Chinese spice, is frequently utilized as a "Qi tonifier" and has been read up for its remedial advantage in treatment of ischemic coronary illness, myocardial dead tissue, cardiovascular breakdown, and help of anginal torment. Clinical examinations have shown that its *in vitro* cell reinforcement action is the component by which it manages the cost of its cardioprotective advantage.

However still in the early formative stages, cardiovascular nanomedicine is probably going to satisfy the serious need for cutting edge development in the determination and treatment of cvds. Unique in relation to ordinary sub-atomic therapeutics, nanomedicine empowers the plan of multi-

part, performing various tasks, multi-secluded specialists that can all the while and exactly distinguish and treat sickness. For instance, we can imagine savvy nano-sensors coordinated in existing inserts, for example, defibrillators, stents or pacemakers that might set off admonitions, or maybe intense arrival of medication, whenever required. One more nanomedicine answer for CVDs could be anticipated for weak plaques; 'click science' or exceptionally controlled cross-connecting techniques focusing on and 'getting' the plaque before resulting AMI without risk of blocking the vessel could be used (Godin et al., 2010).

The positive outcomes from the HCV preliminary (which utilizes LNA-changed antimir against mir122, which is expected for replication of HCV) and late translational exploration offer an exceptionally encouraging point of view on the convenience of mirnas as helpful focuses in CVD. Given the advances in how we might interpret the job of mirnas in cardiovascular physiology and illnesses, it appears to be simply a question of time before new mirna-based remedial methodologies are proposed and tried in clinical settings and, ideally, tracked down successful in the treatment of CVD (Wronska et al., 2015).

Effect of magnesium

Co-administration of magnesium sulphate with doxorubicin effectively corrected changes in the stimulation threshold and contractile force brought on by doxorubicin, according to Khalilzadeh et al. (2018). Treatment of doxorubicin, magnesium sulphate, and normal saline intraperitoneally to male Wistar rats four times a week for two consecutive weeks. Then, electrocardiograms, inotropic tests, and biochemical test were reported as doxorubicin-induced animal mortality was reduced by magnesium sulphate and body weight loss was improved. Additionally, it was found that rats treated with magnesium sulphate had a reduction in the doxorubicin-induced lesions. In doxorubicin-treated rats, magnesium sulphate markedly enhanced Glutathione (GSH).

In conclusion, using a simple PVP-directed self-assembly method, we created a Magnesium hexacyanoferrate nanocatalyst with strong ferrous ion capture and antioxidation properties for cardioprotection during chemotherapy for tumours. The mghcf ncs effectively restored the viability of the cardiomyoblasts damaged by DOX to a greater extent than the much less effective DXZ agent currently used in the clinic, which was demonstrated and distinguished by regulating apoptosis and ferrous ion traffic at both the mrna and protein levels. This was based on the excellent superoxide dismutase and H2O2 decomposition catalytic activities to eliminate cytotoxic radical species. Magnesium hexacyanoferrate nanocatalysts also demonstrated exceptional in vivo cardioprotective activities by reducing cardiotoxicity and negative side effects brought on by DOX contamination without impairing the chemodrug's antitumor effects.

According to Caspi et al. (1993), Mg2+ supplementation affords protection against catecholamine-induced cardiotoxicity under severe (X- and J-adrenergic stimulation of the newborn myocardium. When it comes to congenital heart disease and possible heart donors, when brain trauma and high levels of endogenous catecholamines may lead to Left Ventricle failure, this beneficial effect may be clinically meaningful. This work shows that simultaneous treatment of Mg2+ and high dosages of epinephrine protects against the development of acute cardiotoxicity and LV dysfunction in a model of a stressed neonatal myocardial.

Amitriptyline

Amitriptyline seems to contrast from different antidepressants concentrated on in this series by causing critical weakness of heart conductance in the older. Nonetheless, contrasts in drug levels may mostly make sense of these distinctions, and changes in cardiovascular conductance during treatment with imipramine have been accounted. (Kristiansen, 1961).

An emergency clinic based drug data framework has been utilized to explore a doubt connecting unforeseen demise with the organization of the tricyclic stimulant, amitriptyline, in patients with a finding of cardiovascular sickness. 6 out of 53 patients with cardiovascular sickness who were distinguished by the framework passed on out of nowhere and suddenly after organization of the medication, contrasted and none of 53 control patients matched for sex, age,

analysis, and length of stay in emergency clinic. This high recurrence of surprising passing was not found in patients getting imipramine, nor in patients without heart illness getting amitriptyline. Amitriptyline ought to be involved with alert in patients with heart illness (Coull et al., 1976).

The high recurrence of startling passing was not found in patients without cardiovascular sickness getting amitriptyline nor in patients with heart illness getting imipramine. It was seen that the heart patients getting imipramine involved a geriatric populace possessing extended stay beds though the amitriptyline patients involved intense clinical beds. It is reasoned that amitriptyline ought to be involved with alert in patients with heart illness (coull et al., 1970; Lin et al., 2022).

Results demonstrate that solitary oral organization of amitriptyline and paracetamol alone or consolidated weaken nociception in a tireless torment model. Rehashed dosages of the two medications diminished cciinduced mechanical allodynia. Isobolographic examination shows a practical cooperation between the amitriptyline and paracetamol in the formalin test and CCI model. Moreover, the blend can forestall CCI-prompted WD. Hence, this blend could be useful to treat neuropathic torment in the facility (Garrido-Suárez et al., 2021).

Effect of cucurbitacin on CVDs

The current study offers fresh proof of cucurbitacin I's inhibitory effects on cardiac hypertrophy. Furthermore, Jeong et al. (2015) showed that the reduction of CTGF and its downstream signalling pathways, MAPK and TGF-/Smad signalling, which are desirable targets for the prevention and/or therapy of cardiac disorders, is most likely what mediates these advantageous features. We also suggest cucurbitacin I as a potential therapeutic and preventative measure for ventricular hypertrophy and heart failure.

The current investigation showed that exposure to H2O2 alone lowered the viability of H9c2 cells while pretreatment with Cu I boosted the viability of H2O2-treated cells in a dose-dependent way. Additionally, pretreatment with Cu I in H2O2-induced H9c2 cells demonstrated reduction of ROS generation and enhanced expression of numerous antioxidant proteins (SOD-1, catalase, and GPx). Therefore, according to these findings (Yang et al., 2018), Cu I significantly reduces oxidative stress in H2O2-treated H9c2 cardiomyoblasts. The current investigation reveals

that cucurbitacin B has a marked direct effect on autophagy in both heart and liver, supporting Xiao et al.'s (2017) claim that it is a strong inhibitor of cardiac hypertrophy by targeting the Akt signal pathway and subsequent autophagy.

METHODOLOGY

Objective 1: Synthesis and characterization of cucurbitacin conjugated magnesium nanoparticles.

Cucurbitacin conjugated Magnesium nanoparticle synthesis:

Magnesium oxide nanoparticles will be prepared by precipitation method. 5g of magnesium sulphate (MgSO4) will be dissolved in 100ml of distilled water and 5g of sodium hydroxide (NaOH) pellet will dissolved in 100ml of distilled water. 50ml of magnesium sulfate solution and 50ml of sodium hydroxide solution will be mixed. The solution will be stirred on a magnetic stirrer for 2h. Magnesium hydroxide will be precipitated. The solution will be heated at 100^{o}C for 2 hours in hot air oven. Preparation of MgO nanoparticles (Lidvin et al., 2015). The precursor will be heated in a muffle furnace at 300^{o}C for 3 hours. MgO nanoparticles will be produced. Further MgO will be amine functionalized, then conjugated with Cucurbitacin (Purchased) (Balakriishnan et al., 2016). Nanoparticles will be characterized by SEM, EDX, FTIR, UV-Sepctrosopy.

Objective 2: Evaluation of cardioprotective effects of cucurbitacin conjugated magnesium nanoparticles in *in-vitro* and *in-vivo* system.

***In-vitro* investigation**

- **Cytotoxicity studies**

The cells will be trypsinized and aspirated into a 50ml centrifuge tube. H9C2 cell pellet will obtained by centrifugation at 300 x g. The cell count will be adjusted, using MEM medium containing 10% FBS, such that 200µl of suspension contained approximately 15,000 cells. The cells will be seeded on 96 well plate and incubated for 24 hours in CO_2 incubator at 37°C and5 % CO_2. The test concentration of the MgO and MgO- Cucurbitacin sample under study will be added in different concentrations and incubated for 24 hours under the same conditions. After the incubation MTT will added and observed for the formation of formazan using microplate reader.

- ***In vitro* cardioprotective activity**

In vitro cardioprotective activity will be evaluated by "MTT pretreatment method". H9C2 cardiomyocyte cell lines (will be procured from NCCS, Pune) will used for then cardioprotective activity. The cells will trypsinized and aspirated into a 50ml centrifuge tube. Cell pellet will obtained by centrifugation at 300 x g. The cell count will be adjusted, and subcultured into MEM

medium containing 10% FBS, such that 200µl of suspension contained approximately 15,000 cells. The cells will be seeded on 96 well plate and incubated for 24 hours in CO_2 incubator at 37°C and5 % CO_2. The test concentration of the MgO and MgO- Cucurbitacin under study will added in different concentrations and incubated for 24 hours under same conditions. The media containing MgO and MgO- Cucurbitacin containing media will be removed by aspiration and the cells will be then challenged with cardiotoxin and incubated for 3 hours. After the incubation MTT will be added and observed and analyzed using microplate reader.

***In-vivo* investigation**

- **Approval of Animal Experiments**

All the animal experimental procedures and protocols that will be covered in this study will be reviewed and approved by the Institutional Animal Ethical Committee (IAEC). Ethical guidelines will be strictly followed during all the experiments.

- **Oral Acute Toxicity Studies**

Acute toxicity study was performed according to Organisation for Economic Co-operative and Development guidelines (OECD) No. 423. Albino rats with (n=6) in each groups will be used. Test samples will be administered orally as single doses to rats at different dose levels. Animals were observed individually during the first 30 minutes and periodically during 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total 14 days. At the end of the study the animals were observed for general toxic signs, morphological behaviour and mortality.

- **Induction of Cardiotoxicity in animals**

At the end of treatment period, all the animals, except the normal untreated rats that served as the control group, will be administered cardiotoxin, interaperitoneal injection for two consecutive days on the 31 and 32 day at an interval of 24 h. to induce myocardial injury. After 48 hours rats will anaesthetized, then sacrificed and the hearts will be harvested for biochemical and histological studies.

- **Allocation of groups and experimental Protocol**

The rats will be randomly divided into six groups with (n=6) in each group. Group I, normal animals will receive saline 10ml/kg b.w with standard feed and water to allow ad libitum throughout the experimental period. Group II, the rats will receive cardiotoxin. Group III, rats will

be treated with standard drug along with cardiotoxin and subsequent group animals will receive Mgo, cucurbitacin, cucurbitacin conjugated magnesium nanoparticles respectively along with cardiotoxin.

- **Collection of Blood and Heart Tissues**

At the end experiment, all the animals will be sacrificed by mild ether anaesthesia and the blood sample of each group will be collected and serum will be subjected for estimation of biochemical enzymes like lactate dehydrogenase (LDH), creatine kinase (CK), serum aspartate amino transferase (AST), and the lipid profile of total cholesterol (TC), triglyceride (TG), HDL and LDL. The heart excised immediately and immersed in physiological saline and will be suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer and cut into small pieces. The required amount will be used for estimation of cardiac enzymes of LDH, CK and AST. And part of heart tissue will be used for histopathological studies. And part of heart tissue will be stored in Trizol solution and used for RNA isolation for transcriptomics analysis.

- All experimental Data will be statistically analyzed.

Objective 3: Unmasking the mode of action of cucurbitacin conjugated magnesium nanoparticles through transcriptomics and network analysis.

- **Total RNA Isolation and Quantification**

Experimental heart tissue will be collected and frozen at -80˚c in trizol solution. Total RNA will be isolated from the heart tissue by using Trizol reagent based method. Total RNA will be quantified and quality will be analyzed.

- **cDNA libaray preparation and transcriptome sequencing library**

The paired-end sequencing library will prepared using RNA Library Prep Kit. Cluster generation and sequencing will be conducted on NGS platform through RNA-Seq technology.

- **Transcriptome functional analysis**

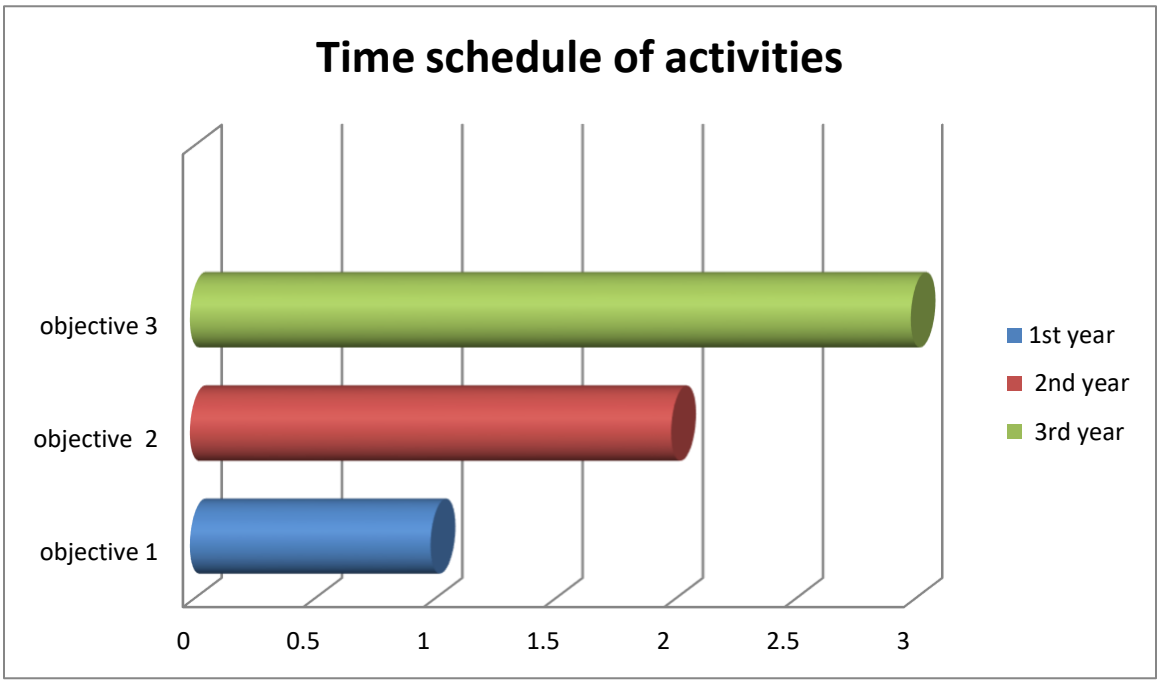
Transcriptome analysis will be carried out for identification of distinct gene transcripts that map to rat genome and display significant differential expression in heart tissue between Control, cardio-intoxicated, standard drug treated and test drug treated. Gene Ontology (GO) analysis will be used to describe the properties of genes that were differentially expressed. The KEGG pathway-

based analysis helps in the understanding of specific functions of the DE genes in certain biological process.

- **Prediction of bottle neck protein:**

Prominently differentially expressed genes will be subjected to gene ontology analysis using G:profiler. Cytoscape will be used for predicting bottleneck protein for cardiotoxicity and cardioprotectivity.

Time schedule of activities giving milestones through bar diagram:



Expected outcome:

Cardioprotective effect of Cucurbitacin conjugated magnesium nanoparticles will be studied. Reports indicate that magnesium nanoparticles elicit neuroprotective property against excitotoxicity. In the proposed investigation we will explore the cardioprotective effects of magnesium nanoparticles and Cucurbitacin conjugated magnesium nanoparticles against cardioexcitotoxicity. Further this investigation will unmask the mode of cardioprotective action of the nanoparticles through transcriptomics and network analysis.

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